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Pratama, Akbar Adjie; van Elsas, Jan Dirk

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## MINIREVIEW

# Gene mobility in microbiomes of the mycosphere and mycorrhizosphere –role of plasmids and bacteriophages

Akbar Adjie Pratama<sup>†</sup> and Jan Dirk van Elsas<sup>\*</sup>

Department of Microbial Ecology – Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

<sup>\*</sup>Corresponding author: Department of Microbial Ecology – Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands. Tel: +31 (0)50 3632151; Fax: +31(0)50 363 2348; E-mail: [j.d.van.elsas@rug.nl](mailto:j.d.van.elsas@rug.nl)

**One sentence summary:** This review examines the state-of-the-art in research of gene mobility across bacteria in mycospheres and mycorrhizospheres, highlighting the roles of plasmids, phages and genomic islands.

**Editor:** Marcus Horn

<sup>†</sup> Akbar Adjie Pratama, <http://orcid.org/0000-0003-1079-744X>

## ABSTRACT

Microbial activity in soil, including horizontal gene transfer (HGT), occurs in soil hot spots and at “hot moments”. Given their capacities to explore soil for nutrients, soil fungi (associated or not with plant roots) can act as (1) selectors of myco(rhizo)sphere-adapted organisms and (2) accelerators of HGT processes across the cell populations that are locally present. This minireview critically examines our current understanding of the drivers of gene mobility in the myco(rhizo)sphere. We place a special focus on the role of two major groups of gene mobility agents, i.e. plasmids and bacteriophages. With respect to plasmids, there is mounting evidence that broad-host-range (IncP-1 $\beta$  and PromA group) plasmids are prominent drivers of gene mobility across mycosphere inhabitants. A role of IncP-1 $\beta$  plasmids in Fe uptake processes has been revealed. Moreover, a screening of typical mycosphere-inhabiting *Paraburkholderia* spp. revealed carriage of integrated plasmids, next to prophages, that presumably confer fitness enhancements. In particular, functions involved in biofilm formation and nutrient uptake were thus identified. The potential of the respective gene mobility agents to promote the movement of such genes is critically examined.

**Keywords:** mycosphere; horizontal gene transfer; plasmids; bacteriophages; evolution

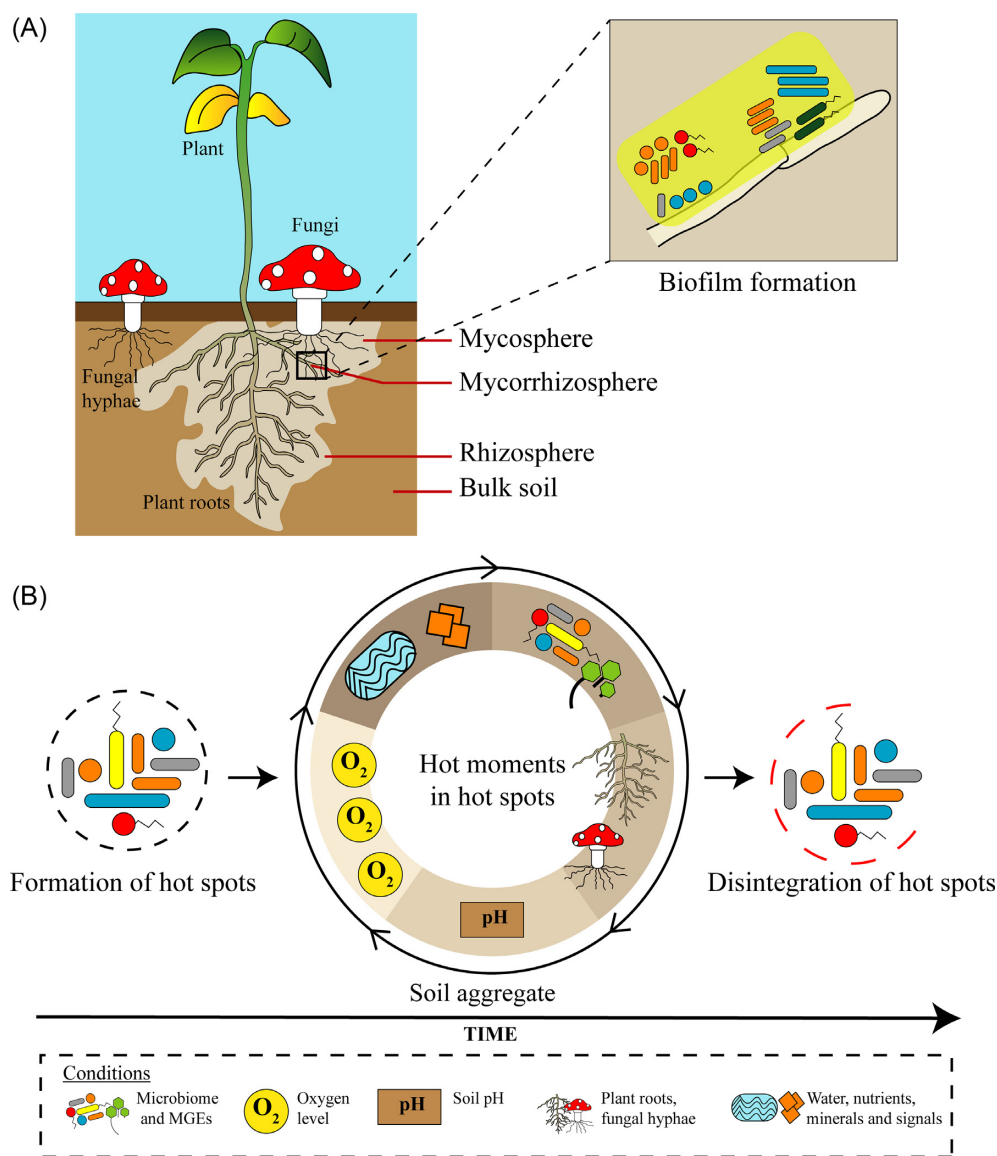
## INTRODUCTION

The living soil is crucial for all life on Earth, and this highlights the relevance of soil microbiomes for key life support (C and N cycling) functions (van Elsas et al. 2019). Next to being spatially heterogeneous, soil microbiomes are also remarkably dynamic in time. Thus, rises and falls, in terms of abundance and activity, of the diverse populations that make up these microbiomes

are commonly observed (van Elsas et al. 2019). Varying levels and gradients of, e.g., nutrients, minerals, pH and water in the spatially heterogeneous soil matrix are at the basis of the variability in soil microbiomes (Fierer 2017). In fact, many bacteria in soil commonly occur in a state of nutrient deprivation (leading to dormancy), and they will only incidentally become active. Based on this finding, the concept of “hot moments”

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**Figure 1.** Depiction of the mycosphere and mycorrhizosphere. (A) Heterogeneity of soil creates microhabitats; ecologically relevant processes mainly occur in the small volumes of soil denoted hot spots. These are the most active parts of the soil, in which microbes accelerate metabolic rates and evolutionary processes. Additionally, the enhancement of spatial proximity between the local cell populations in soil hot spots may culminate in the formation of biofilms or similar aggregations. Hot spots include mycospheres (i.e. soil surrounding fungal hyphae), mycorrhizospheres (i.e. soil surrounding intertwined plant roots and fungal hyphae) and rhizospheres (i.e. soil surrounding plant roots). (B) Soil hot spots form as a result of hot moments (i.e. high rates of short-lived microbial activity) of microbial populations, related to soil aggregates, redox potential, nutrient contents and biotic binding agents (e.g. fungal hyphae and roots). Once established (inside hot spots), cells increase metabolic processes and interactions. Consequently, accelerated microbial activities spur genetic exchanges and thus evolution occurs. Upon disintegration and disappearance of the hot spots, formerly enclosed microbiomes mend with the gene pool hosts in the surrounding populations.

(short time spans characterized by enhanced microbial activities) was recently coined by Kuzyakov and Blagodatskaya (2015). Such hot moments come about as a result of the emergence of local conditions favoring metabolism and growth. The end of such hot moments may be gradual, and the established activity hot spots may still maintain certain levels of bacterial activity (Kuzyakov and Blagodatskaya 2015; see Fig. 1). Microbial life in soil has, thus, been characterized as obeying a “feast and famine” type of existence, with the *feast* parts being equivalent to the relatively short hot moments and the *famine* parts often being quite extended.

We here define mycospheres, in a ‘loose’ sense, as the zones in soil that are influenced by fungal hyphae, whereas mycorrhizospheres can be defined as the soil zones in the vicinity of

plant roots that are influenced by mycorrhizal fungi (Sarand et al. 1999; Zhang et al. 2014a). Both habitats are typical examples of microsites where activity hot spots and moments (Kuzyakov and Blagodatskaya 2015) occur, in a highly dynamic manner. How do these come about? Regarding the former habitat, saprotrophic fungi explore the soil for nutrients, establishing novel nutrient-rich microhabitats. With respect to the latter, it has been estimated that around ~90% of all terrestrial plant species are associated with soil fungi to form mycorrhizas (Selosse et al. 2006), mostly either ectomycorrhizae (EM) or arbuscular mycorrhizae (AM). For bacteria dwelling in soil, carbon thus becomes available from the photosynthates that are transferred from the host plants via the roots to the associated mycorrhiza (Pickles et al. 2017).

Interestingly, Hannula *et al.* (2012) identified not only mycorrhizal but also saprotrophic fungi, such as *Rhizopus oryzae* and *Rhodocybe mundula*, as being involved in the acquisition of recently-formed photosynthate in the rhizosphere of potato (*Solanum tuberosum*). Their evidence pointed to the often overlooked fact that such fungi are key early responders to photosynthate carbon when that becomes available in the rhizosphere. Thus, rhizospheres often contain considerable areas that yield possibilities for activity hot spots/hot moments.

It should be noted that both mycospheres and mycorrhizospheres constitute highly dynamic habitats. The microsites in these habitats are characterized by rather limited lifespans, as fungal tissue first builds up (thus offering dynamic sites for colonization by adapted organisms), increasingly releasing compounds that may attract or feed these, and in a later stage senesces and eventually dies out (Brabcová *et al.* 2016). The whole lifespan of a local mycosphere may range from days to weeks, and that of a mycorrhizosphere up to several months, depending on the conditions and success of colonization (Warmink, Nazir and van Elsas 2009; see also Fig. 1b). In the light of the variability in space and time, the bacterial dwellers of these two habitats most likely require a suite of intricate metabolic, uptake and stress response systems in order to cope with the local dynamism in terms of nutrient availability and conditions (Table 1). As can be seen from the Table, in both habitats conditions will emerge that favor bacteria endowed with capacities to colonize and acquire local nutrients.

Another key issue with respect to the bacteria living in soil is their frequent inability—due to the barriers posed by the solid soil matrix—to reach other organisms, or a suitable substrate, even if these are present only millimeters away. Thus, bacterial cells are thought to be generally confined to the soil microhabitats they dwell in, except when transport agents are available (Berthold *et al.* 2016; Yang, Oliveira da Rocha Calixto and van Elsas 2018). In contrast, most soil fungi—in particular saprotrophic ones—explore the soil for available nutrients, in particular carbonaceous compounds that yield sources of carbon and energy. Their network structures enable them to move around substances (e.g. bound carbon) and so carbon sources that are required for growth can be furnished to local bacteria (Haq *et al.* 2018). Moreover, the hyphal networks can also translocate bacterial cells through soil (Warmink and van Elsas 2009; Berthold *et al.* 2016).

Overall, the highly dynamic conditions in mycospheres and mycorrhizospheres will pose requirements for strong adaptability of bacteria in order for the latter to establish, colonize the local microhabitat, grow and survive. In other words, ‘survival of the fittest’ is likely to drive the evolution of the fungal-associated bacteria. Which agents affect the evolution of these locally adaptive organisms? Clearly, both agents that enable “optimization” (Torsvik and Thingstad 2007) and “flexibilization” (Nielsen and van Elsas 2019) confer eco-evolutionary advantages, often related to capacities required to acquire locally available carbon and energy sources, as well as other essential nutrients, next to those enabling to withstand local stressors (Thomas and Nielsen 2005).

In this minireview, we briefly examine the selective forces exerted on bacteria in mycospheres and mycorrhizospheres, focusing on the agents of the horizontal gene transfer (HGT) processes in these. We also address relevant evidence regarding the prevalence and/or role of mobile genetic elements (MGEs) in the rhizosphere, in the context of the potential role of fungi in this habitat.

## EFFECTS OF THE MYCOSPHERE AND MYCORRHIZOSPHERE ON SOIL BACTERIA

### Characteristics of the mycosphere and mycorrhizosphere as selective habitats in soil

As argued in the foregoing, mycospheres and mycorrhizospheres constitute matrices that enable local bacteria to interact with the fungal host as well as with each other. Table 1 depicts the key conditions in these microhabitats, as well as the resulting effects on the surrounding microbiota. First, the active hyphae of fungi in soil often supply (release) small carbonaceous compounds that support the growth of associated bacteria. Thus, compounds such as: maltose, trehalose, L-rhamnose, D-sorbitol, D-glucuronic acid, p-hydroxy-phenylacetic acid, succinamic acid, glucuronamide, L-alanine amine, L-orithine, glycerol, mannitol, oxalic acid, acetic acid, formic acid, citric acid, fumaric acid and CO<sub>2</sub> have all been shown to be released at fungal surfaces (Frey-Klett *et al.* 2011; Churchland and Grayston 2014; Haq *et al.* 2018). Moreover, hyphae of particular fungi are known to produce inhibitory (i.e. bactericidal) or signaling compounds (Deveau *et al.* 2018), such as may have been the case in the study by Sengeløv, Kovvalchuk and Sørensen (2000). On another notice, fungal hyphae may locally affect soil structure and/or change (raise) the soil pH (Nazir *et al.* 2010), the latter effect potentially establishing a hospitable environment for the local bacteria. Finally, fungal hyphae in soil often establish and maintain a liquid film around them, reflecting their capacity of hydraulic redistribution (Gühr *et al.* 2015). The mycelial networks in soil can thus serve as concentration/transport modules for soil bacteria (Berthold *et al.* 2016; Yang, Oliveira da Rocha Calixto and van Elsas 2018). Clearly, the extent to which a mycosphere or mycorrhizosphere is conducive to bacterial colonization and activity depends on the characteristics, in terms of the dynamic growth, physiological adaptation and eventual death of the fungal host (Warmink, Nazir and van Elsas 2009). In many cases, the effects of released carbonaceous compounds, next to the pH effect, will tend to create hospitable environments in which bacteria can thrive. However havoc may be incited if antagonistic compounds prevail. As an example, particular saprotrophic basidiomycetes, i.e. agaricoid fungal species, produce antimicrobial substances that inhibit the growth of Gram-positive bacteria, making their surroundings inhospitable for these bacteria (de Boer *et al.* 2005). Moreover, wood rot fungi like *Hypholoma fasciculare*, *Phanerochaete velutina*, *Phallus impudicus*, *Resinicium bicolor* and *Phlebiella vaga* sometimes also strongly inhibit bacteria (El Arieibi *et al.* 2016). All of these fungi were found to produce sesquiterpene compounds (e.g. cadinene, longifolene,  $\beta$ -patchoulene and caryophyllene oxide), which are known to have antibacterial properties (El Arieibi *et al.* 2016).

The frequently observed cases in which bacterial abundances and processes in mycospheres/mycorrhizospheres are promoted as compared to those in the surrounding bulk soil (Warmink and van Elsas 2008; Churchland and Grayston 2014; Zhang *et al.* 2014a; Kuzyakov and Blagodatskaya 2015) are thought to reflect the emergence of local activity hotspots (characterized by raised nutrient levels, a moderate pH and the presence of life-supporting water levels). These factors trigger increases in local cell densities, which may lead to shifted microbiome structures and enhanced activities (Frey-Klett *et al.* 2011; Deveau *et al.* 2018).

**Table 1.** The effects of mycospheres/mycorrhizospheres on (A) local conditions and (B) bacteriomes, in comparison to bulk soil.

Parameter		Mycosphere			Mycorrhizosphere		
		Main effect	Specific remarks	References	Main effect	Specific remarks	References
<b>A. Effect on local conditions</b>							
Main compounds	C	Enhanced level of carbonaceous compounds and mineralization	Compounds found: maltose, trehalose, L-rhamnose, D-sorbitol, D-glucuronic acid, p-hydroxy-phenylacetic acid, succinamic acid, glucuronamide, L-alanin amine, L-ornithine, glycerol, mannitol, oxalic acid, acetic acid, formic acid, citric acid, fumaric acid and CO <sub>2</sub>	Warmink, Nazir and van Elsas 2009; Boersma et al. 2010; Frey-Klett et al. 2014; Guhr et al. 2015	Enhanced level of carbonaceous compounds and mineralization	Compounds found: oxalic acid, formic acid, citric acid, malonic acid, gluconic acid, succinic acid, protocatechoic acid, isocitric acid, malate and CO <sub>2</sub>	Churchland and Grayston 2014; Guhr et al. 2015
	N	Indirect stimulatory influence	Through bacterial N <sub>2</sub> fixation, N assimilation-NH <sub>4</sub> <sup>+</sup>	Holz et al. 2016	Indirect stimulatory influence	Through bacterial N <sub>2</sub> fixation, N assimilation-NH <sub>4</sub> <sup>+</sup>	Holz et al. 2016
	P	Enhanced available P levels	Through PO <sub>4</sub> <sup>3-</sup> mineralization, transport, P solubilization and CO <sub>2</sub> release	Rashid et al. 2016	Enhanced P levels	Through PO <sub>4</sub> <sup>3-</sup> mechanisms, such as uptake and translocation of P	Battini et al. 2017s
	Fe	Modulation of Fe levels	Through Fe mobilization	Rashid et al. 2016	Modulation of Fe levels	Root exudates maintain soil Fe concentration	Rashid et al. 2016
Miscellaneous inorganic compounds		Increased level of mineral weathering	Increased Si and K, and decreased Al level.	Calvaruso et al. 2009	Increased level of mineral weathering	Increase of Si was observed in Norway spruce ( <i>Picea abies</i> [L.] Karst) and oak ( <i>Quercus sessiliflora</i> Smith) mycorrhizospheres. Increase of K observed in oak.	Calvaruso et al. 2009
pH		Modulation of mycosphere pH (often a small raise)	Mycosphere in microcosm soil shows pH increase along with fungal growth, i.e., pH from 4.6 to 5.0.	Nazir et al. 2010; Yang, Oliveira da Rocha Calixto and van Elsas 2018	Modulation of mycosphere pH	Mycorrhizosphere pH raised towards neutral.	Carrino-Kyker et al. 2016
Water		Altering of mycosphere water regime	Fungal hyphae alter soil pore structure and water distribution, also impacting nutrient and cell mobilization.	Guhr et al. 2015	Altering of water regime	Mycorrhizospheres shape soil hot spots by altering soil structures, water distribution and soil nutrient mobilization.	Kuzyakov and Blagodatskaya 2015; Rillig, Muller and Lehmann 2017
Salinity		Indirect effect on soil salinity	Increase in hyphal networks alleviates salt stress. Mechanism: facilitation enhancing nutrient acquisition.	Liu et al. 2015	Alleviating saline stress	By increasing the colonization by AM fungi. Mechanism: enhanced nutrient acquisition.	Liu et al. 2015
<b>B. Effect on local bacteriomes</b>							
Bacteriome density		Enhancement of local density	Raise of up to 10 <sup>8</sup> CFU per gram soil in <i>Lyophyllum</i> sp. strain Karsten mycosphere. Specifically, 10 <sup>7</sup> –10 <sup>8</sup> CFU of <i>Variovorax paradoxus</i> and <i>Paraburkholderia terrae</i> .	Nazir et al. 2010; Zhang, Yang and van Elsas 2016	Increases	Raise of up to 10 <sup>8</sup> CFU per gram soil in the <i>Carex arenaria</i> mycorrhizosphere	De Boer et al. 2015
Bacteriome structure		Modulation of structure due to mycosphere-specific conditions	Different fungi select different bacterial populations. <i>Laccaria proxima</i> increases the abundance of <i>Pseudomonas</i> and <i>Paraburkholderia</i> spp. <i>Tylopus felleus</i> increases <i>Pseudomonas</i> and <i>Ewingella</i> populations	Warmink, Nazir and van Elsas 2009	Selection of particular bacteria	Exudates select specific bacterial populations. For example, legume mycorrhizosphere ( <i>Gigaspora margarita</i> ) increases <i>Paraburkholderia</i> spp.	Brabcová et al. 2016
Cell-to-cell interactions and activity <sup>a</sup>		Enhancement of intensity of interactions	Genetic interactions are promoted	Zhang et al. 2014a	Enhancement	Increase in bacterial interactions and activities due to the increase of available nutrients	Kuzyakov and Blagodatskaya 2015



Table 1. Continued

Parameter	Mycosphere			Mycorrhizosphere		
	Main effect	Specific remarks	References	Main effect	Specific remarks	References
Predation	Lowering of predation pressure for fungal-associated cells in biofilm.	Bacterial biofilms around hyphae protect against predators, e.g., protozoa	Nazir et al. 2010	Lowering of predation pressure	Decrease of protozoan numbers, possibly due to antimicrobials in the mycorrhizosphere; biofilms protect against protozoa.	Wamberg et al. 2003
Antimicrobial activity and toxicity	Increases	Fungal-associated bacteria produce, e.g., phenazines, chitinases and proteases.  Fungi produce antibiotics that inhibit diverse bacteria.	De Boer et al. 2005	Increases	Mycorrhizospheres select <i>Pseudomonas fluorescens</i> able to produce, e.g., hydrogen cyanide (HCN), that protects against phytopathogens	Frey-Klett et al. 2014

<sup>a</sup>Refers to bacterial growth, metabolic processes, nutrient acquisition, responses to environmental factors (this regards the presence of fungal and plant roots), interaction towards other bacteria, to the extent of gene transfer.

### Cell-to-cell interactions

That the mycosphere as well as the mycorrhizosphere can establish activity hot spots in soil has been shown by several authors (Nurmiaho-Lassila et al. 1997; Frey-Klett, Garbaye and Tarkka 2007; Warmink and van Elsas 2008; Zhang et al. 2014a). For instance, in a study performed in our lab, the abundance of *Pseudomonas* populations was found to increase dramatically at the base of the EM fungus *Laccaria proxima* (Warmink and van Elsas 2008), and also particular *Sphingomonas* and *Paraburkholderia* types revealed shifted communities. Figure 1 gives an impression of the stimulatory effects of soil fungi on hypha-associated bacteria. The end result is often an enhanced spatial proximity between the local cell populations, which may culminate in the formation of biofilms or similar aggregations (Berthold et al. 2016; Haq et al. 2016). In theory, such enhanced cell-to-cell proximity, equivalent to raised cell densities (expressed per unit soil volume) will enhance the frequencies of (genetic) interactions between the cells. In more detail, the microcolonies or biofilms at the hyphal surfaces incite, across the constituent cells, varying processes of approximation, colonization, signal exchange and growth. In such processes, the key MGEs that are released, such as phages, plasmids or other cellular DNA, will—due to the mass action effect—have increased chances to be in contact with neighbouring cells. As the fungal surface itself is subjected to phases of hyphal build-up, maturation and senescence, and local conditions may thus continually change, the genetic interactions in the microhabitat are not at all straightforward. Thus, the fate and evolution of locally-adaptive traits is reigned by the system's conditions, regarding both the gene transfer rates and the selective effects exerted by the system on the recipient cell populations (Zhang et al. 2014a).

### Selection of fungal-associated bacteria, in particular *Paraburkholderia* spp.

The notion of soil fungi, including saprotrophs and mycorrhiza, as “selectors” of particular bacterial types is relatively ‘old’ (Warmink, Nazir and van Elsas 2009; Berthold et al. 2016; Pent, Pöldmaa and Bahram 2017). It was recently confirmed by Brabcová et al. (2016), who showed that *Pseudomonas* (next to *Ewingella*) was selectively favored at the EM fungus *Tylophylus*

*felleus*. Moreover, different fungi may select a suite of similar bacteria (Warmink, Nazir and van Elsas 2009), in addition to diverse ones. As an example, the mycosphere of the EM fungus *L. proxima* was found to primarily select *Paraburkholderia*, *Dyella* and *Pseudomonas* spp., while another EM fungus, *Russula exalbicans*, favored different members of the *Sphingomonadaceae* (Boersma et al. 2009). In other, more recent, work, *Paraburkholderia*, *Bacillus* and *Paenibacillus* spp., next to mycobacteria, were found to be abundant at the EM fungus *Tricholoma matsutake*, while (in addition to *Paraburkholderia*) *Bradyrhizobium* and *Acidobacteria* spp. were abundant in the mycospheres of *Marasmius* and *Lepiotaceae* spp. (Halsey, de Cássia Pereira e Silva and Andreote 2016). Also, Nguyen and Bruns (2015) recently showed *Paraburkholderia* and *Rhizobium* spp. to be major groups associated with the EM fungus *Pinus muricata*. *Paraburkholderia*, next to *Dyella*, *Pseudomonas* and *Sphingomonas* spp., were also highly prevalent at *Meliniomyces variabilis*, *Russula* sp. 6 GJ-2013b and *Paxillus involutus* (all at *Pinus sylvestris* roots; Marupakula, Mahmood and Finlay 2016). In other recent work, Johnston, Boddy and Weightman (2016) also revealed the prime occurrence of *Paraburkholderia* types as co-migrators with the hyphae of several wood-rot fungi.

From the aforementioned studies, some specific organisms consistently emerged as potentially “universal” fungal-associated bacteria. In other words, these appear to constitute bacterial groups that typically interact with soil fungi. Indeed, in many studies, *Paraburkholderia* spp. (often within the *P. terrae*/*P. hospita* cluster) have been encountered as prime and consistent colonizers of fungal tissue in soil. In a key laboratory study, Haq et al. (2016) recently provided evidence for the high affinity and chemotactic movement of one such organism, *P. terrae* BS001, toward the hyphae of the soil saprotrophs *Lyophyllum* sp. strain Karsten and *Trichoderma asperellum*. Interestingly, an attractant role for several small carbonaceous compounds present at fungal surfaces, e.g. glycerol and oxalic acid, was found (Haq et al. 2016). Here, we will place a focus on mycosphere-derived *Paraburkholderia* as the model/paradigm organism, in order to critically examine the potential of HGT as a mechanism that enhances the adaptability of these organisms to conditions of the mycosphere.

## AGENTS OF GENE MOBILITY IN THE MYCOSPHERE AND MYCORRHIZOSPHERE

In a range of studies, the classical HGT mechanisms transformation, transduction and conjugation have been amply shown to be at the basis of the flexibility and adaptability of the genomes of soil bacteria (Nielsen and van Elsas 2019). All three processes require cellular activities, being transformation dependent on particular host genes (including competence factors), transduction on bacteriophage and specific host genes that need to be expressed by the host, and conjugation on systems (often on plasmids, e.g. type-IV secretion systems - T4SS) that allow the construction of cytoplasmic bridges between the donor and recipient cells that interconnect. As the frequencies of all three mechanisms are raised by cell-to-cell proximity as well as cellular activity (Thomas and Nielsen 2005), we hypothesize that they are all potentially promoted in those mycospheres and mycorrhizospheres that confer conditions propitious for cell-to-cell approximations and activities (Zhang et al. 2014a).

A first requirement for host adaptability, irrespective of the types of MGEs, is that particular ephemeral adaptive traits ("accessory" genes that confer fitness advantages under particular ecological conditions, yet are dispensable most of the time) are sufficiently present in the local gene pools. An additional, but not indispensable, asset would be the linkage of such genes to MGEs (Zhang et al. 2014a). Clearly, such linkage facilitates gene transfer, potentially assuring an immediate fitness enhancement of a recipient organism following transfer (if the relevant expression systems are compatible with the recipient organism).

Taking the linkage criterion into our investigations (see Soucy, Huang and Gogarten 2015), we will in the following section examine selected *Paraburkholderia* strains, in addition to other ones (obtained from mycospheres) for MGEs. We first address the occurrence and role of plasmids as separate genetic entities, and then the respective genomes for inserted MGEs, as the paradigm of adaptive agents in the mycosphere and mycorrhizosphere.

## PLASMIDS AS GENE MOBILITY AGENTS IN THE MYCOSPHERE AND MYCORRHIZOSPHERE

### Role of plasmids as genetic flexibility agents

Plasmids are here taken as relatively small (< 200 kb) genetic elements that can replicate independently from the chromosomes of their hosts. Most plasmids are either self-transferable, mobilizable and/or retromobilizable and so can move from their host to other bacteria. Regarding the host ranges of plasmids, these vary from narrow (basically confined to a species or genus) to extremely broad (even crossing the Gram boundary). Moreover, some plasmids may occasionally integrate into a recipient chromosome, giving rise to genomic islands. Such integrative events, or the eventual transfer of a transposon from an incoming plasmid to a novel host, are particularly relevant in cases where plasmids have broader transfer than replication ranges (Nielsen and van Elsas 2019). The finding of plasmids (with broad host ranges), such as those of the PromA group, without any discernable accessory genes (termed "cryptic" plasmids; Tauch et al. 2002; Heuer et al. 2004) has given rise to the hypothesis that these may serve the purpose of population-scale gene transfer flexibility. The broad host range character of such plasmids is of prime eco-evolutionary relevance, as it will allow the rapid spread of genetic novelty throughout a major part of a local microbiome.

Possibly, bacteria accept and maintain plasmids as tools to, thus, create standing genetic variation within a population, allowing particular constituents of the population to quickly respond to the (adverse) conditions that may occur in the environment. Over time, insertions of relevant genes may thus occur at particular sites. Some scientists will consider this particular plasmid feature to be a token of their "selfishness". In other words, such plasmids use local (recipient) cell populations to their own benefit, as microbial carrier "donkeys", and the combination of the plasmid as a gene transfer vehicle, the accessory gene, and the novel host will occasionally constitute an ecologically-viable form under particular conditions.

Clearly, examinations of extant mycosphere / mycorrhizosphere plasmids, along with their accessory gene loads, will yield a legacy of past events of gene gain (and loss) as well as selection. Unfortunately, extensive overviews of this kind have not been made so far.

That transfer of plasmids from a current host to fitter host organisms (yielding novel gene/host combinations) is at the basis of their persistence in bacterial populations was already suggested by Bergstrom, Lipsitch and Levin (2000). According to their theory, plasmids are indeed driven by events of periodic selection of novel combinations of plasmid, accessory gene and host. In the next section, we address to what extent mycospheres and mycorrhizospheres enable plasmids to persist and evolve across the fungal-associated bacterial populations.

### Plasmids in the mycosphere and mycorrhizosphere: occurrence and putative roles

Initial studies on the potential role of plasmids in the mycosphere were performed by Sarand et al. (1998, 1999). The roles of the biodegradative TOL plasmid pWW0 (117 kb) (Sarand et al. 1998), and of a similar plasmid (approximately 150 kb) (Sarand et al. 1999) were tested in a *Pseudomonas fluorescens* host at the EM fungus *Suillus bovinus* associated with *Pinus sylvestris* roots. Both plasmids had capacities to degrade *m*-toluate and *m*-xylene (next to benzene, ethylbenzene and *o*-, *m*- and *p*- xylene), and so they might enhance host fitness in systems containing *m*-toluate. The data revealed that, in such systems containing the plasmid-endowed *P. fluorescens* with *S. bovinus*, the *m*-toluate levels decreased by 29–50% (day 1) to 58–79% (day 4), as compared to the control (fungus alone) (Sarand et al. 1999). Thus, maintaining the plasmid was beneficial to the bacterial host and fungus alike. In a recent study in agar microcosms set up so as to measure the effect of bacterial motility (high, medium and low) on plasmid pWW0 transfer between *Pseudomonas putida* strains, pronounced emergence of transconjugants in microcosms with (*Pythium ultimum*) mycelium was found (Berthold et al. 2016). This suggested the existence of preferential dispersal pathways and enhancement of HGT in the presence of fungal mycelium.

### Importance of broad-host-range character of plasmids

Broad-host-range plasmids constitute key connectors of genomes across suites of separate bacterial lineages (Tauch et al. 2002; Sen et al. 2011; Shintani et al. 2014; Klümper et al. 2015). That a scenario of rapid plasmid spread is actually likely to occur under transfer-conducive conditions, was demonstrated in a study by Klümper et al. (2015). In their study, the authors showed that with *P. putida* KT2440, *Escherichia coli* MG1655 and *Kluyvera* sp. as donor strains, the transfer of IncP-1 and PromA- type plasmids to diverse hosts from agricultural soils present in soil microbiomes, when the latter are exposed to

"crowded" and nutrient-rich conditions, is a realistic scenario. In their experiment on agar plates, transfers were found to a large suite of diverse bacterial phyla, both Gram-negative and -positive, i.e. ( $\alpha$ - $\epsilon$ ) Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, *Fusobacter*, Gemmatimonadetes, Planctomycetes, spirochaetes, candidate division TM7 and Verucomicrobia. Arguably, these plasmids can also interconnect bacterial with Eukaryote genomes (Zhang et al. 2014a). Given the propensity of mycospheres and mycorrhizospheres to enhance local cell densities of diverse bacterial types, we posit that broad-host-range plasmids are key gene mobility agents in these natural mycosphere bacteriomes. Several recent studies have indeed implicated the highly promiscuous IncP-1 $\beta$  as well as PromA group plasmids as potentially important agents of HGT in mycospheres. In the following, we place emphasis on each of these two plasmid groups.

### IncP-1 $\beta$ plasmids

Recent evidence has identified plasmids of the IncP-1 $\beta$  group as major MGEs in rhizosphere bacteriomes (Bziuk et al. 2016). Thus, using molecular quantification of the IncP-1 $\beta$  marker gene *korB*, enrichments of such plasmids were found in the rhizospheres of lettuce and tomato. Furthermore, triparental matings, using an IncQ plasmid as the tracer, captured high levels of IncP-1 $\beta$  plasmids from these habitats (Nour et al. 2017). Whereas this finding can be taken as evidence for an IncP-1 $\beta$  selective effect of plant roots, a possible role for fungi of the rhizosphere (and so mycospheres and/or mycorrhizospheres) cannot be excluded. Indeed, as described in Zhang et al. (2015), IncP-1 $\beta$  plasmids were typically present in several *Variovorax paradoxus* isolates obtained from the mycosphere of *L. proxima*. In other work, they were also encountered in the microbiomes of various other mycospheres, such as those of *Russula*, *Inocybe* and *Ampulloclitocybe* spp. (Zhang et al. 2014b). Selected plasmids, i.e. pHB44 and pBS64, were found to stimulate the production of biofilms in their *V. paradoxus* BS64 hosts as compared to plasmid-less variants (Zhang et al. 2015). Moreover, a presumed iron uptake system present on the IncP-1 $\beta$  plasmid pHB44 was found to assist the fitness of the host organism, *P. terrae* strain BS001, in the mycosphere of *Lyophyllum* sp. strain Karsten, but only under low-Fe conditions (Zhang, Yang and van Elsas 2016). In contrast, in soil with sufficient Fe, plasmid carriage resulted in a lowering of host fitness, revealing a predicted effect of an enhanced energy or other burden on the host cell (Fig. 2). Finally, the in-mycosphere transfer of plasmid pHB44 from *P. terrae* BS001 to the co-inhabitant *V. paradoxus* BS64 was also strongly enhanced under the low-Fe conditions as compared to higher Fe levels. Overall, these data yield evidence for the tenet that, indeed, plasmids of the IncP-1 $\beta$  type constitute agents of genetic flexibility, across taxonomic borders that enable mycosphere-dwelling bacteria to quickly take profit of key plasmid-borne functions that are instantaneously required to survive.

### PromA type plasmids

Whereas the first (cryptic) PromA group plasmid, pIPO2, was obtained from a rhizosphere soil (van Elsas et al. 1998; Tauch et al. 2002), another highly homologous PromA group plasmid, pTer331, was found in a mycosphere-isolated *Collimonas fungivorans* strain (Mela et al. 2008). Furthermore, the early work on the prototype PromA plasmid pIPO2 (Tauch et al. 2002) pointed to a prevalence of PromA target sequences in rhizospheres of diverse plant species. As argued in the foregoing, a role for

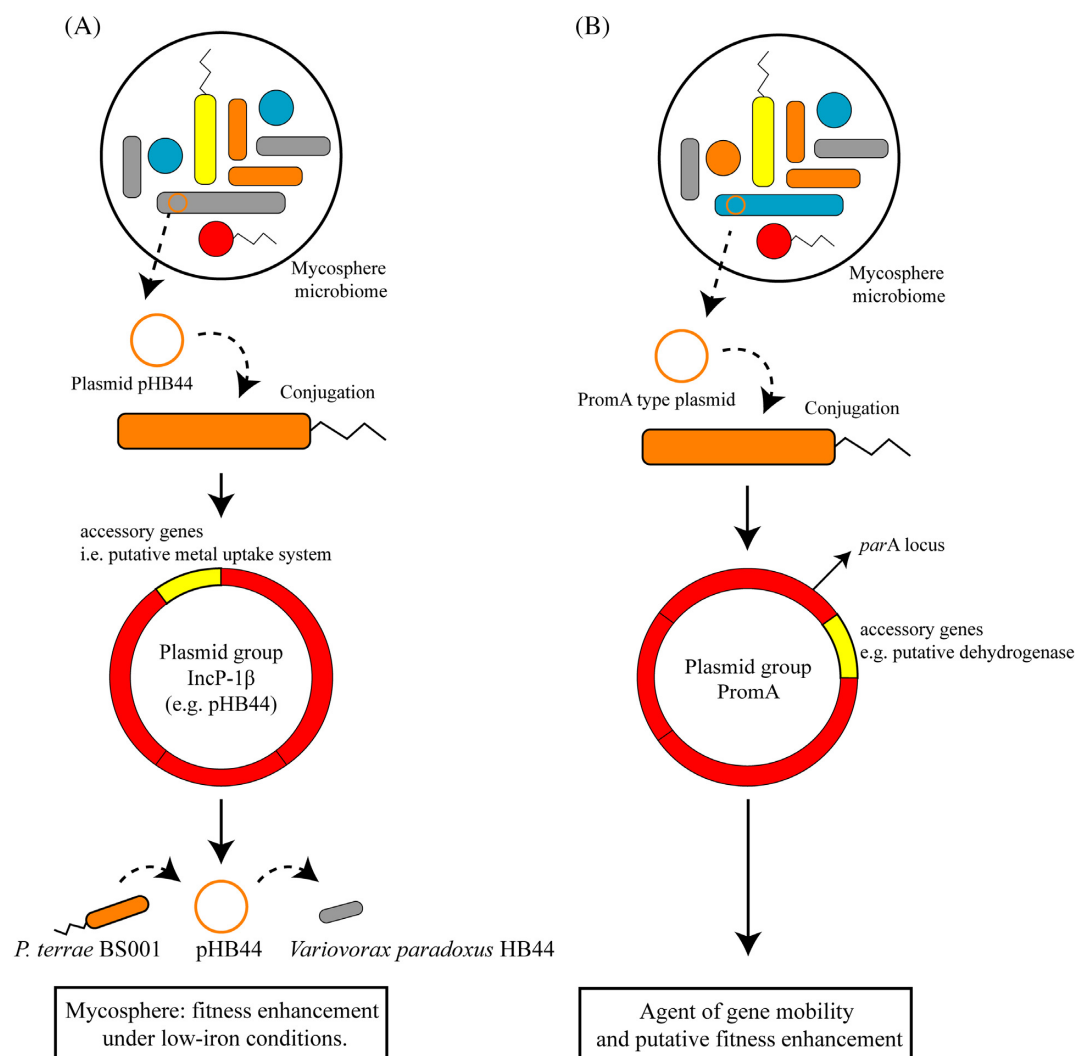
fungi drivers in these rhizospheres cannot be excluded. On the basis of the *repA* gene, Zhang et al. (2014b) found PromA sequences to be highly prevalent at the EM fungi *Russula*, *Inocybe* and *Ampulloclitocybe* spp., as well as directly in the forest floor soil. Moreover, PromA type plasmids were also found to transfer frequently into hosts used in triparental exogenous isolation experiments (Zhang et al. 2014b). However, these mycosphere plasmids have been given the epithet cryptic, as finding clear functional genes on them has been difficult. Interestingly, the *parA* locus on PromA plasmids was identified as being conducive to insertions of functional genes (Schneiker et al. 2001; Dias et al. 2018), and so a polymerase chain reaction-based detection system of such insertions was designed (Dias et al. 2018). Indeed, in a recent study three typical insertions into the *parA* locus of PromA group plasmids were encountered in DNA derived from the mycospheres of different fungi. All of these insertions were different from those typically found in two bulk soils (which included genes for an esterase and an endo-1,4-beta-xylanase), one resembling a gene for a putative dehydrogenase (from an uncultured bacterium) (Dias et al. 2018). In future work, it will be a challenge to determine the extent to which PromA type plasmids are indeed important carriers, as well as genetic flexibility agents, of relevant mycosphere-adaptive traits across members of mycosphere microbiomes.

## ANALYSIS OF THE GENOMES OF MYCOSPHERE-COMPETENT *Paraburkholderia* spp. FOR GENE MOBILITY AGENTS

### RGPs – genomic islands with features of integrated MGEs

Regions of genomic plasticity (RGPs) are here defined as genomic regions that are acquired through HGT, often containing genes that encode behaviour/fitness-modulating functions, including pathogenicity and symbiosis (Dobrindt et al. 2004). Bacteria that have access to the horizontal gene pool often contain large numbers of RGPs, as well as other mobile regions (Dobrindt et al. 2004). In an initial screening, Haq et al. (2014) reported a total of 16% of the *P. terrae* BS001 genome to be inside RGPs (detected by the Microscope platform, about 79 regions). In this organism, RGP76 (70.4 kb), RGP24 (31.45 kb), RGP61 (51.15 kb) and RGP62 (53.22 kb) were reported to carry typical plasmid-related genes, suggesting their plasmid-derived ancestry. Most conspicuously, the 70.4-kb RGP76 contained an entire T4SS with a full set of archetypical functional genes, thus revealing clear features of a plasmid with full transfer potential. RGP76 further contained genes for a DNA replication protein (*repB*), for stable maintenance and partitioning (*korC*, *klaA*, *parA*, *parB*, *parD* and *parE*) and for mobilization/transfer (*mobB* and *mobC*), next to multiple genes for transposases (e.g. *tnpA*, *tnpB* and *insH*) and integrases. Further evidence for the contention that the RGP76 region was plasmid-derived was provided by the finding that its T4SS was highly syntenous to that of the (functional) megaplasmid pBPHY02 of *Paraburkholderia phymatum* STM815. Six additional RGPs found in the strain BS001 genome (RGP47 - 46.2 kb; RGP20 - 48.1 kb; RGP25 - 46.4 kb; RGP38 - 53.8 kb; RGP49 - 96.2 kb and RGP53 - 102.9 kb) carried genes for transposases and integrases, also suggesting past events of gene mobility. Moreover, RGP53 carried genes for a siderophore-binding protein, an invading plasmid antigen J (*ipaJ*) and a type-III effector protein (*hopJ*); this points to functions relevant for host fitness in the soil/mycosphere.





**Figure 2.** Roles of two groups of broad-host-range plasmids in the mycosphere. (A) The IncP-1 $\beta$  plasmid pHB44 enhances *Paraburkholderia terrae* BS001 fitness under low-iron conditions in the mycosphere and also transfers to *Variovorax paradoxus* HB44 under these conditions (Zhang et al. 2014a, 2014b, 2015). (B) The broad-host-range PromA group plasmids act as agents of highly promiscuous and frequent HGT, including insertions of accessory genes into the *parA* locus (van Elsas et al. 1998; Zhang et al. 2015; Dias et al. 2018).

Additional analyses of the genomes of the mycospheric *P. terrae* strains BS007, BS110 and BS437 (Pratama et al. 2017), along with the *Paraburkholderia* type strains *P. terrae* DSM 17804<sup>T</sup>, *P. hospita* DSM 17164<sup>T</sup> and *P. caribensis* DSM 13236<sup>T</sup>, showed the presence of T4SSs in all genomes (incomplete in BS007) but that of *P. caribensis* DSM 13236<sup>T</sup>. In *P. terrae* DSM 17804<sup>T</sup> and *P. hospita* DSM 17164<sup>T</sup>, the T4SSs were located inside RGP97 (157 kb) and RGP99 (520 kb), respectively. In all cases, complete sets of T4SS genes, i.e. *virB1* to *virB11*, next to *VirD4*, were found to be flanked by typical (integrative/conjugative) plasmid-related genes (see Table 2). Furthermore, other conserved plasmidic regions (i.e. plasmid-related genes, such as those encoding the antitoxins ParE1 and ParB) were also found, next to diverse insertions (i.e. accessory genes) and deletions (Fig. 3). Some of these accessory genes (Table 2) potentially encode traits that may ephemerally enhance fitness of the host in soil. For example, genes for the biosynthesis of glutamate, tyrosine, threonine and methionine, of antibiotics (phenazine), for degradative functions (of ethylbenzene and chlorocatechol), for chitinase, next to genes for additional metabolic steps (of butanoate, amino acids, nitrogen

and methane) were encountered. Furthermore, genes for antibiotic resistance, stress responses and an antitoxin protein were also found (see Table 2). These analyses showed that: (i) RGP are very common across the examined *Paraburkholderia* genomes, which is consistent with the versatile and plastic lifestyles of these organisms, and (ii) presumed gene mobility through the T4SSs was conserved among these *Paraburkholderia* spp., with the notion that the identified regions were variable across the strains (see Table 2). Thus, the presumed integrated plasmids were unique per strain/species, as also found by Manna, Park and Seo (2019). A recent study of the genomes of *P. phymatum*, *P. caribensis* and *P. phenoliruptrix*, for which a relationship with plants was cogitated, showed the presence of RGPs endowed with genes for plant-symbiotic and -growth-promoting roles (e.g. *nod*, *nif*, ACC deaminase) (Manna, Park and Seo 2019). Together, these data indicate that, along evolutionary time, the genomes of the mycosphere-dwelling *Paraburkholderia* species that were examined, in addition to those of the type strains, have been amply subjected to diverse invasions by plasmid-like elements of diverse sizes, i.e. 60-kb up to 500-kb. It is likely that

**Table 2.** Regions of genomic plasticity (RGPs) containing a T4SS in mycosphere-derived and other *Paraburkholderia* spp.

Strain [Acc. No] <sup>a</sup>	Source	RGP <sup>b</sup>	Size (bp)	# genes	Typical genes [predicted functions]	MGE <sup>c</sup> genes	Reference
<i>P. terrae</i> BS001 [AKAU00000000]	Mycosphere of <i>Lyophyllum</i> sp. strain Karsten	RGP76	70 422	89	Presumed relevant accessory genes: <ul style="list-style-type: none"> <li>• O-acetyl homoserine sulphydrylase (biosynthesis of fusaric acid),</li> <li>• Transcriptional activator protein PhzR (antibiotic biosynthesis),</li> <li>• 1,4-<math>\alpha</math>-glucan branching enzyme GlgB (glycogen biosynthesis)</li> </ul> Other genes: <ul style="list-style-type: none"> <li>• Cytoplasmic membrane protein,</li> <li>• Molybdopterin binding aldehyde oxidase and xanthine dehydrogenase (oxidoreductase),</li> <li>• 3-phosphoshikimate 1-carboxyvinyltransferase (aromatic amino acid biosynthesis),</li> <li>• DNA-directed RNA polymerase II subunit RPB2,</li> <li>• L-methionine gamma-lyase (seleno amino acid metabolism),</li> <li>• Cobyrinate a,c-diamide synthase (anaerobic biosynthetic pathway of adenosyl cobalamin),</li> <li>• Transcriptional repressor protein KorC (DNA binding).</li> </ul>	6 transposases, 9 integrases, <i>ParA</i> , <i>ParB</i> , <i>ParD</i> , <i>ParE</i> , <i>korC</i> , <i>repB</i> , <i>MobB</i> , <i>MobC</i> , <i>KlcA</i> .	Haq et al. 2014
<i>P. terrae</i> BS110 [NFVD00000000]	Mycosphere of <i>Laccaria proxima</i>	RGP45	116 299	159	Presumed relevant accessory genes: <ul style="list-style-type: none"> <li>• N-ethyl maleimide reductase (aromatic compound degradation),</li> <li>• Superoxide dismutase- <i>sodF</i> (stress response),</li> <li>• Poly-beta-hydroxybutyrate polymerase (transferase activity, transferring acyl groups, poly-hydroxybutyrate biosynthetic process),</li> <li>• Putative tautomerase K2</li> </ul> Other genes: <ul style="list-style-type: none"> <li>• Pyridoxal 4-dehydrogenase(B6 metabolism),</li> <li>• Alanine dehydrogenase,</li> <li>• Glutamate synthase,</li> <li>• Citrate lyase (fatty acid metabolism),</li> <li>• Mannitol dehydrogenase,</li> <li>• L-galactonate oxidoreductase,</li> <li>• Cobyrinate a,c-diamide synthase (anaerobic biosynthetic pathway of adenosylcobalamin)</li> </ul>	6 transposases, 8 integrases (2 phage integrases), toxin <i>ParE1</i> , antitoxin <i>ParD</i> , <i>ParB</i> , <i>RepB</i>	Pratama et al. 2017
<i>P. terrae</i> BS437 [NFVC00000000]	Mycosphere of <i>Lyophyllum</i> sp. strain Karsten	RGP25	151 786	184	Presumed relevant accessory genes: <ul style="list-style-type: none"> <li>• Chitin deacetylase (Hydrolase),</li> <li>• Xylanase (xylan degradation),</li> </ul> Aminoglycoside phosphotransferase (antibiotic resistance), <ul style="list-style-type: none"> <li>• Nitroreductase</li> <li>• Oxygen-insensitive NADPH nitroreductase</li> </ul> Other genes: <ul style="list-style-type: none"> <li>• 3-methyl-2-oxobutanoate hydroxymethyltransferase (pantothenate and CoA biosynthesis),</li> <li>• Phosphate acetyltransferase (taurine and hypotaurine metabolism, pyruvate metabolism and propanoate metabolism),</li> <li>• Stress protein <i>UspA</i> (universal stress protein),</li> <li>• Acetyl-CoA synthetase,</li> <li>• Alcohol dehydrogenase,</li> <li>• Putative phosphoketolase (carbohydrate metabolism),</li> <li>• Amidohydrolase 2 (hydrolase),</li> <li>• 4-hydroxythreonine-4-phosphate dehydrogenase (vitamin B<sub>6</sub> metabolism)</li> </ul>	8 transposases, plasmid pBRH01 gene of unknown function	Pratama et al. 2017

Table 2. Continued

Strain [Acc. No] <sup>a</sup>	Source	RGP <sup>b</sup>	Size (bp)	# genes	Typical genes [predicted functions]	MGE <sup>c</sup> genes	Reference
<i>P. terrae</i> DSM 17804 <sup>T</sup> [CP026111-CP026114]	Forest soil	RGP97	157 478	174	Presumed relevant accessory genes: <ul style="list-style-type: none"> <li>• Phthalate 4,5-dioxygenase oxygenase subunit (Xenobiotic metabolism),</li> <li>• Rhodocoxin reductase (degradation of the thiocarbamate herbicide),</li> <li>• (S)-1-Phenylethanol dehydrogenase (aromatic degradation),</li> <li>• Ferredoxin-NAD(P)(+) reductase <i>fdr</i> (aromatic carbazole metabolism),</li> <li>• Ferredoxin <i>CarAc</i> (carbazole metabolism)</li> </ul> Other genes: <ul style="list-style-type: none"> <li>• Dehydrogenase,</li> <li>• Amidohydrolase,</li> <li>• 2Fe-2S ferredoxin (electron transport),</li> <li>• Fumarylpyruvate hydrolase (bacteria degradation of 5-substituted salicylates),</li> <li>• Gentisate 1,2-dioxygenase (amino acid tyrosine metabolism),</li> <li>• Methylmalonate-semialdehyde dehydrogenase, oxidoreductase protein (inositol metabolism, valine, leucine and isoleucine degradation and propanoate metabolism),</li> <li>• Aspartate ammonia-lyase (alanine and aspartate metabolism and nitrogen metabolism),</li> <li>• Aminoacylase (urea cycling)</li> </ul>	3 transposases, Integrase family protein, <i>ParB</i> family protein	Yang et al. 2006
<i>P. hospita</i> DSM 17164 <sup>T</sup> [CP026105-CP026110]	Soil	RGP99	419 946	689	Presumed relevant accessory genes: <ul style="list-style-type: none"> <li>• Protein <i>YdeP</i> (possible acid resistance),</li> <li>• Manganese catalase (antioxidant enzyme),</li> <li>• S-(hydroxymethyl)glutathione dehydrogenase (methane metabolism),</li> <li>• Zinc-dependent sulfurtransferase <i>sufUSCB</i></li> <li>• Glyoxalase/bleomycin resistance protein/dioxygenase,</li> <li>• Phasin family protein (carbon and energy reservation),</li> <li>• Carbonic anhydrase (interconvert carbon dioxide and bicarbonate),</li> </ul> Other genes: <ul style="list-style-type: none"> <li>• Amino acid (glutamine synthetase, glutamate biosynthesis, cysteine metabolism),</li> <li>• Exonuclease,</li> <li>• Oxidase,</li> <li>• D-erythronate dehydrogenase (carbohydrate metabolic/ oxidoreductase activity),</li> </ul>	<i>ParB</i> , 5 integrases, 20 transposases, 2 IS3 element protein <i>InsE</i> , 3 IS2 insertion element transposase <i>InsAB</i> , Intron <i>gplI</i> , one plasmid stabilization system, two KpLE2 phage-like element, toxin <i>ParE1</i> , two antitoxin <i>ParD</i>	Goris et al. 2002

<sup>a</sup>NCBI accession number; <sup>b</sup>The genomes are hosted at MicroScope platform (<https://www.genoscope.cns.fr/agc/microscope/home/>) (last accessed 2019), where RGPs analyses were performed; <sup>c</sup>MGE: mobile genetic element. Data fully published in Pratama et al. (in prep).

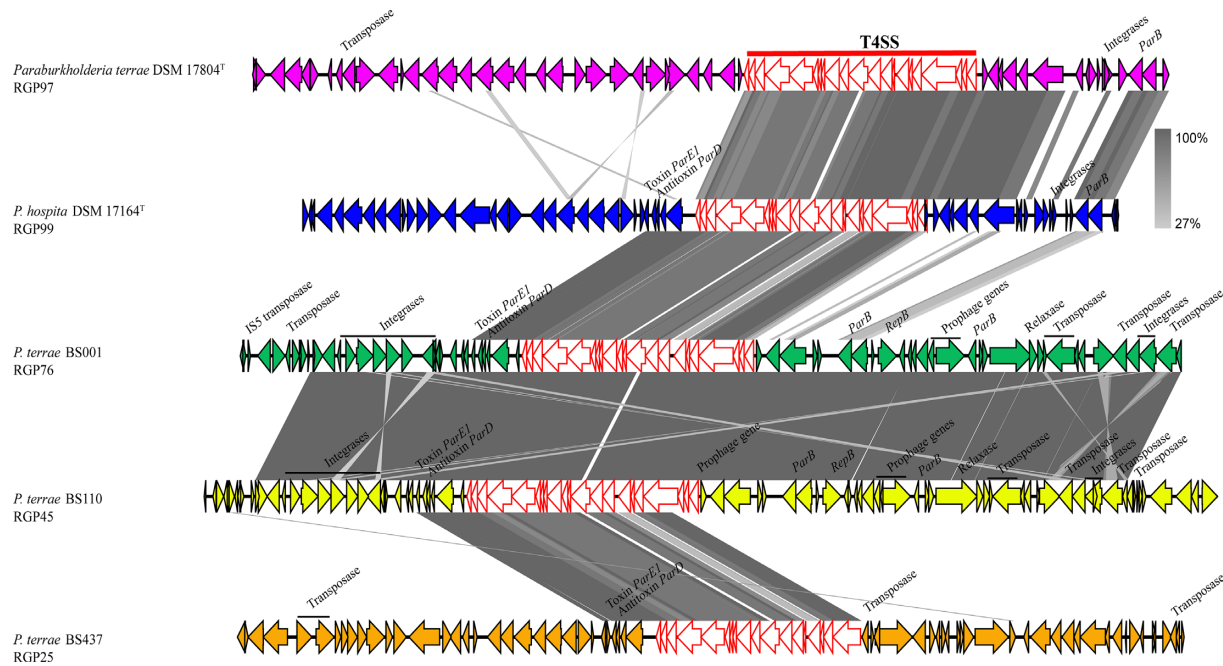
different events of gene loss have also occurred. The resulting inserts were found to be mosaic, and diverse, in structure. It will be a challenge to determine what types of selective pressures have been involved in favoring the emergence and persistence of such elements in the respective bacterial genomes.

### Prophages and phages

Our current knowledge with respect to prophage prevalence in mycosphere and soil dwellers is still scarce. Not only do we largely ignore the extent of phage prevalence, but also do we understand little about their roles and functions. To what extent are prophages anchored in host genomes able to exert tasks like population control or HGT? With respect to soil bacteria, a classical study established a role of phages as transfer vehicles of

small tetracycline resistance plasmids across soil-derived *Bacillus* strains (van Elsas and Pereira 1987). Moreover, other studies on such phages (van Elsas and Penido 1982; Quesada, Soriano and Espinosa-Urgel 2012) have offered snapshots of potentially vast soil phage communities.

With respect to the model mycosphere bacterium *Paraburkholderia*, recent compelling evidence has identified a potential role of their (pro)phages. The initial finding of a typical phage gene, i.e. an integrase, in the genome of *P. terrae* BS001 (Haq et al. 2014) led to the discovery of 'remnant' prophage regions, most of which had been subjected to deletion bias (i.e. the loss of viral reproduction and structural genes). More recent work showed that prophages indeed occur across the genomes of diverse mycosphere-derived *P. terrae* and *P. hospita* strains (Pratama and van Elsas 2017; Pratama,



**Figure 3.** Analysis of (30–40) genes flanking the T4SS (type-IV secretion system) across the genomes of three mycosphere-associated *Paraburkholderia* strains, next to two “type strains”. The commonalities of large regions versus the mosaic structures of other regions are shown. The percentages of similarity (100–27%) are shown in the grey-shared bars.

Chaib De Mares and van Elsas 2018). In detail, such prophages were omnipresent and occupied up to 4% of genome space, specifically, in the mycosphere (*Trichoderma harzianum*) -derived *Paraburkholderia* sp. MF2-27. Most regions identified as prophage, however, did not contain all genes necessary for completion of a lytic cycle (Pratama, Chaib De Mares and van Elsas 2018). Therefore, notwithstanding technical constraints, we here posit that, following the events of prophage insertion, multiple gene losses occurred in these organisms, in the context of the highly-dynamic soil and mycosphere sites inhabited by them.

A number of questions arises here. What is the cost of carrying these prophages? To what extent do they offer immediate benefit to the host? What are those benefits? What is the evolutionary consequence of carrying these prophages? What can we say, for instance, about the *Paraburkholderia* sp. MF2-27 prophages found in multiple copies in the genome (Pratama, Chaib De Mares and van Elsas 2018), with respect to its habitat (the *Trichoderma harzianum* mycosphere), in comparison to other mycospheres? Theoretically, lysogenic phages may benefit their host through: (i) “engineering” their host genome, (ii) changing host physiology by new genes, (iii) facilitation of gene mobilization through transduction and (iv) controlling competitor bacteria following release of phage (Howard-Varona et al. 2017). Additionally, prophages promote diversification in bacterial hosts, via integration of prophage encoded-genes (Ohnishi, Kurokawa and Hayashi 2001). Undoubtedly, carrying prophages imposes benefit-harm trade-offs to the bacterial hosts. Concerning the latter, energy costs due to transcription of additional genes and lowering of smoothness of biochemical processes due to prophage integration may occur (Casjens 2003; Obeng, Pratama and van Elsas 2016). With respect to the former, carrying prophages may allow hosts to cope with adverse environments (Wang et al. 2010), such as the pressure posed by similar lytic phages from the outside. Here, the multiple

prophages infesting *Paraburkholderia* sp. MF2-27 are interesting, raising questions about the true benefits of carrying such prophages versus their potential harm.

In mycosphere and soil habitats, the phage-bacterium interaction dynamics, and hence phage activity and lysogenic lifestyle, may be related to phage:bacterium ratio's (PBRs; in other studies denoted as VBRs [virus:bacterium ratio's]). These PBRs, defined as the relative abundance of phage particles compared to that of bacterial cells, have been proposed as proxies of viral significance in a given ecosystem (Williamson, Radosevich and Wommack 2005). Although no data are as yet available for mycospheres/mycorrhizospheres, rhizospheres have revealed values (e.g. 0.27) below those of bulk soils (e.g. 4.68 [Swanson et al. 2009] and 8.20 in hyper-arid desert soils [Zablocki, Adriaenssens and Cowan 2015]). Possibly, the phage lysis-lysogeny decision is connected to local host cell densities (see Silpe and Bassler 2019), and hence PBR values might be reduced in mycospheres / mycorrhizospheres. Interestingly, in mycosphere-inhabiting bacteria other than *Paraburkholderia*, considerable prophage regions, up to 13% of the genomes were encountered (Pratama and van Elsas 2017). Hence, we posit that lysogeny is common across mycosphere and soil dwellers; furthermore, the extent to which the phages affect their hosts in the mycosphere depends on local events of activation of phage cycle and other genes, as discussed in the following.

### Prophages of *P. terrae* and their presumed roles: Φ437 as the paradigm

To the best of our knowledge, prophage Φ437 represents the first phage that was detected in a mycosphere-inhabiting *Paraburkholderia* species (Pratama and van Elsas 2017). The entire 54-kb prophage (containing the minimal set of phage genes) found in the *P. terrae* strain BS437 genome, denoted Φ437, was released upon mitomycin C induction (Pratama and van Elsas 2017). It was later found to be also spontaneously released by



the host cells, and hence questions were raised as to the occurrence of this phenomenon in the mycosphere. Here, we propose two scenarios by which phage  $\Phi 437$  may affect its host in the mycosphere (see Fig. 4). First, prophage-carried “moron” (more-on DNA) gene encoded proteins may confer fitness advantages to host cells in the form of, for instance, auxiliary metabolic genes (Breitbart et al. 2018). Interestingly, one such moron gene was found to be present in  $\Phi 437$ ; it was predicted to encode a gene similar to the *amrZ* gene (PSI-BLAST-P 55% homology, 79% coverage; Phyre<sup>2</sup> analyses 55% homology, 80% coverage, 100% confidence value). This gene is linked to a transcription factor (Pratama and van Elsas 2017) in *Pseudomonas aeruginosa*, where it regulates transcription/repression, primarily of *algD*, the first gene of the alginate biosynthesis operon (involved in bacterial biofilm formation). Thus, *AmrZ* is thought to be involved in enhanced biofilm formation. Moreover, it also controls genes involved in type IV pili (T4P) and twitching motility, and it represses *flaQ*, an activator of flagellar expression (Tart, Blanks and Wozniak 2006). Interestingly, the alginate biosynthetic system was also found to be present in the BS437 genome (Pratama et al. 2017). It is worth to note that, among the *Paraburkholderia* genomes tested, phage  $\Phi 437$  was only found in *P. terrae* BS437, suggesting that it offers a unique accessory mechanism that enhances host biofilm formation. In the second scenario, host biofilm formation may be modulated by spontaneously released phage  $\Phi 437$ , which may cause local holes (and so channels) in the biofilm (Pratama and van Elsas 2017). This is in line with other studies that have pinpointed prophage-mediated lysis as the cause of the release of crucial biofilm-promoting factors, i.e. extrachromosomal DNA and exopolysaccharides (Gödeke et al. 2011; Secor et al. 2015). We hypothesize that phage  $\Phi 437$  may have such a stimulatory role in *P. terrae* BS437 as well.

In a screening for phage  $\Phi 437$  sequences in microbiomes derived from the mycospheres of *Russula ochroleuca* and *R. emetica*, next to corresponding bulk soil, such sequences were consistently found only in bacteriomes derived from the two mycospheres (Pratama and van Elsas 2018). This finding suggested the presence of susceptible *P. terrae* cells and a key role of  $\Phi 437$  in the respective mycospheres, but not in the bulk soil. Interestingly, a viral contig representing another phage, denoted VC14, from populations derived from non-mycosphere soil and predicted to infect *Paraburkholderia* spp., was found to contain a gene encoding a phasin-like protein. PSI-BLASTP analysis of the (translated) gene showed 94% homology and 94% coverage to a phasin element of *Burkholderia* sp. GAS332 as well as *P. xenovorans* (59% homology and 92% coverage). In *Pseudomonas oleovorans* GPo1, phasin (*phaC1*) was predicted to advance host growth by encoding medium-chain-length polyhydroxyalkanoate synthase under excess carbon and limiting nitrogen conditions (Prieto et al. 1999). These conditions may be equivalent to those typically found in region in forest floors. This led to the hypothesis that – indeed – genes for such accessory functions might be prevalent in the (phage) horizontal gene pool. In addition, other novel auxiliary metabolic genes/morons typically found in the phage contigs from the mycosphere included genes for glyoxalase/dioxygenase superfamily proteins (involved in catabolism of aromatics) (Pratama and van Elsas 2018). Moreover, novel auxiliary metabolic genes/morons related to soil carbon cycling were also found, e.g. genes for glycoside hydrolases, next to those for chitinase, peptidoglycan lyase and polygalacturonidase (Pratama and van Elsas 2018). Glycoside hydrolases are involved in degradation of polymers like cellulose, hemicellulose, chitin and/or amylase. Chitin, for example, is a component of the fungal cell wall, and so the presence of chitinase

genes indicated the involvement of phages as carriers of genes for mycosphere degradation processes. These initial insights into mycosphere viromes open up a ‘pandora box’ of unexplored ‘flexible functions’ that play roles in these soil hotspots.

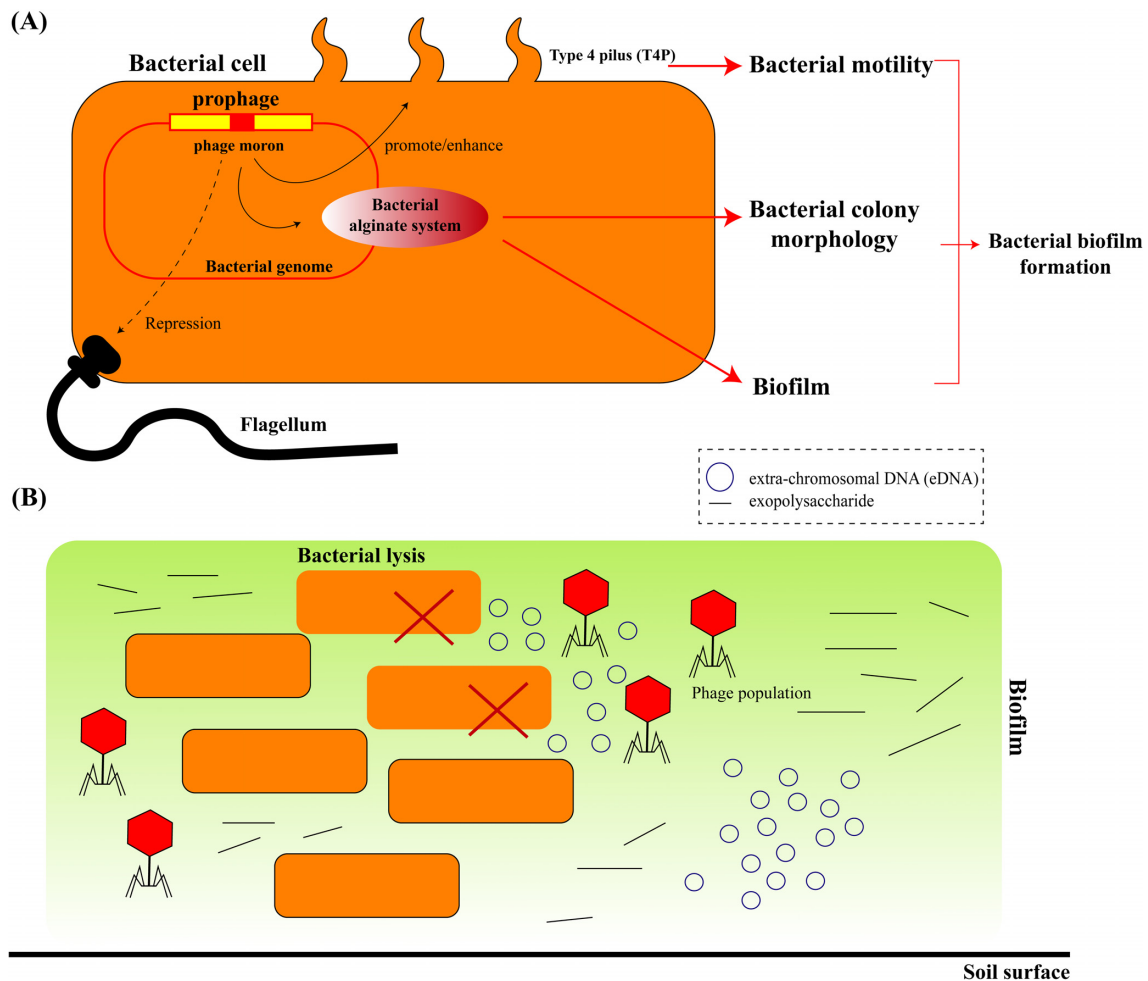
## OUTLOOK

As argued in a previous review (Zhang et al. 2014a), the mycosphere (and mycorrhizosphere) established in soil by, respectively, saprotrophic and mycorrhizal fungi, act to provide both hot spots and hot moments for the activity of soil bacteria. The occurrence of such sites and time spans of enhanced cell density and activity at soil fungi is clearly propitious to HGT processes across local bacteria, much like was previously demonstrated for the rhizosphere (van Elsas et al. 1986; Deveau et al. 2018). However, a note of caution should be placed here, as mycospheres and mycorrhizospheres do differ in their degree of hospitability for soil bacteria. This may range from being extremely hospitable by, for instance, the provision of colonizable surfaces, nutrients and a hospitable pH (allowing large cell densities to build up), to extremely inhospitable, for instance by secretion of antibiotic compounds or lowering the pH to inhospitable levels, e.g. below 5.0. Here, we selected *Paraburkholderia* of the *terrae/hospita* species cluster as the model or reference mycosphere organisms for analysis of the presence and relevance of MGEs as well as RGP. Clearly, although there is overwhelming evidence that these organisms are mycosphere-competent, we basically ignore other parts of their lifestyle and so the data discussed herein need to be regarded with a note of caution. Moreover, other mycosphere-competent organisms may have evolved by parallel routes, and so the analyses may be organismal type-confined.

A hospitable mycosphere or mycorrhizosphere should be viewed as an arena in which cells are in continuous search for available substrate. Competition for this or other substrate is likely to be fierce, and cells that are able to grow and divide will do so in close connection or proximity to other cells, which promotes occasional cell-to-cell interactions. Such active and spatially proximal cells are likely to become involved in HGT processes. Hence, in this context, one may envision a role for plasmids and phages in this competitive life modus. The effects of such HGT processes are exacerbated as the products of HGT constitute potentially strongly-selected evolutionary/adaptive assets that enable mycosphere inhabitants to be ecologically successful. In this scenario, even if transfer rates are local and low-frequency, any adaptive trait (which may be of only ephemeral value), can be quickly shared across local hosts. However, as soon as these elements have turned obsolete, for instance due to shifted conditions, they may be deleted, e.g. by the processes underlying deletion bias or plasmid loss. This fast removal of transiently-required genetic information may thus reduce any metabolic or other burden posed on the host cell populations.

As argued in the foregoing, plasmids and phages may also play roles as ‘physical’ agents that modulate biofilm quality and so may promote biofilm health and strength. Given the relevance of bacterial biofilms in the mycosphere for bacterial survival, we postulate this physical effect to be a second important ‘population-level’ driver of fitness in the mycosphere. As a paucity of knowledge exists on this interesting topic, key research into it is encouraged.

Finally, in the age of massive gathering of microbiome data by soil -omics approaches, the mycosphere has been



**Figure 4.** Proposed role of bacteriophages in the mycosphere and/or the mycorrhizosphere. **(A)** Mycosphere-inhabiting bacteria, e.g., *Paraburkholderia terrae* BS437 carrying prophage  $\phi$ 437, potentially modulate biofilm formation through the prophage-borne transcriptional regulator encoded by the *amrZ*-like gene, which regulates biofilm formation via the alginate system and the type four pilus. **(B)** Spontaneous release of  $\phi$ 437 particles will lyse local host cells in the biofilm. In such local spots, exo-polysaccharides and eDNA (extra-chromosomal DNA) may contribute to the structure of the biofilm.

greatly ignored. It is exactly at the level of the mycosphere/mycorrhizosphere soil hot spots, and at hot moments in these, that the greatest value of these technologies can be found. Future work on mycosphere omics ("mycospheromics") requires an *a priori* consideration of research aims and objectives, which ideally lie in fostering our understanding of the local interactions such as those addressed in this review.

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